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A Drug-Drug Interaction Crystallizes a New Entry Point into the UPR

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In the current issue of *Molecular Cell*, Sicheri and Ron (Wiseman et al. 2010) identify a small molecule, the flavonol quercetin, as an activator of yeast IRE1, a key regulator of the endoplasmic reticulum (ER) unfolded protein response pathway (UPR).

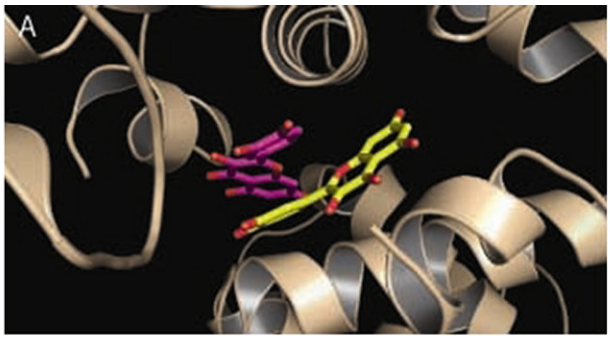
Small-molecule screens are enticing because they offer the dual potential to reveal new biological insights and represent a first step toward discovering therapeutics. In this context, the work by Sicheri and Ron in the current issue is particularly remarkable (Wiseman et al., 2010). It reveals both a new potential drug-binding site on IRE1, a key regulator of the unfolded protein response pathway (UPR), and by virtue of the highly unusual binding mode of the ligand (two drug molecules bind in one protein pocket), their work may offer new avenues for attacking other challenging drug targets.

The unfolded protein response coordinates protein-folding status in the endoplasmic reticulum (ER) and cellular transcription and translation to balance the cell's ability to fold proteins and their production (Ron and Walter, 2007). The ER-localized membrane protein, IRE1 (Inositol Requirement 1), is the most conserved member of the UPR pathway. IRE1 contains an ER luminal domain that senses unfolded proteins, a cytoplasmic portion that contains a kinase domain, and an RNase domain. The RNase domain takes part in a very specific cleavage reaction of an RNA transcript (*HAC1* or *XBP1* in yeast or metazoans, respectively). This IRE1-mediated RNA cleavage reaction initiates an unconventional splicing event leading to production of the XBP1 transcription factor, which results in expression of a large array of transcripts important for protein folding, such as chaperones. The UPR thus represents a concise signaling pathway with IRE1 as a critical sensor of unfolded proteins and the key regulator of the cell's capacity to fold proteins.

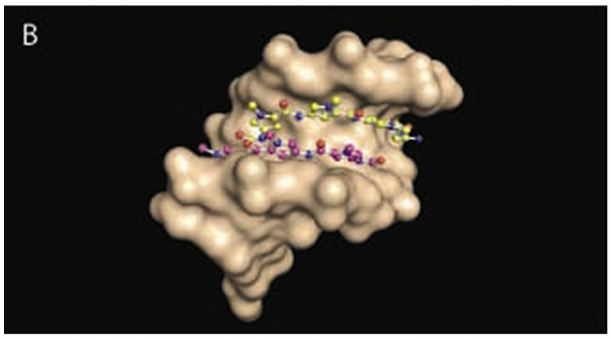
The UPR has become an attractive pathway for drug discovery (Hetz and Glimcher, 2009), as increased XBP1 protein expression has been shown to produce multiple myeloma-like phenotypes in mouse models, IRE1 mutations have been found in large-scale cancer genome screens, decreases in XBP1 levels have been implicated in retinitis pigmentosa, and hypomorphic alleles of XBP1 have been associated with inflammatory bowel disease. These disease associations have led to the search for drugs to modulate the RNase activity of IRE1.

Recently, two crystal structures of the cytoplasmic domains of IRE1 were solved, providing insight into the mechanism of activation of XBP1 splicing (Lee et al., 2008, Korennykh et al., 2009). These studies and others (Papa et al., 2003) demonstrated how ligands that bind to the kinase domain ATP site can induce dimerization and oligomerization of IRE1, leading to the activation of the RNase domain. The communication between the ligand occupancy of the kinase domain and the RNase opened the door to drug development because ligands targeting kinase active sites abound in chemical libraries.

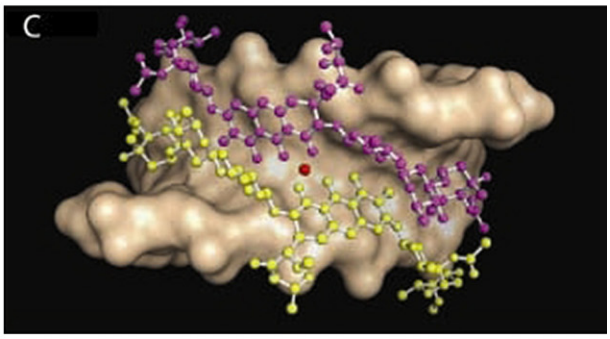
With this backdrop, Sicheri and Ron searched through a library of small molecules for those that could activate or inhibit the RNase activity of IRE1. The most potent "hit" molecule was a flavonol, quercetin, a natural product with



Quercetin-IRE1 Complex



Distamycin-DNA Complex



Chromomycin-DNA Complex

Figure 1. X-Ray Crystallographic Structures of Several Examples of Drug-Drug Dimers Found in Complex with a Protein IRE1 or Duplex DNA
(A–C) Quercetin dimer at the IRE1 dimer interface (A), 2:1 distamycin:DNA complex (PDB ID: 378D) (Mitra et al., 1999) (B), and 2:1 chromomycin:DNA complex (PDB ID: 1VAQ) (Hou et al., 2004) (C). Protein in (A) and DNA in (B) and (C) are shown in ribbon or surface representation. In each panel, the two identical ligands are colored differently, with the carbon atoms in one molecule in yellow and in the other, purple. In (A) and (B), the noncarbon atoms oxygen and nitrogen are shown in red and blue, respectively. In (C), all atoms of each ligand are colored purple or yellow, with the exception of Mg^{2+}, which is shown as a red sphere.

extensive history as a kinase inhibitor. Quercetin is a polyhydroxylated flavinoid that binds to many protein kinases. Careful titration of quercetin with and without the nucleotide site activator ADP suggested that the hit ligand quercetin was not binding to the previously identified nucleotide site in the kinase domain. Here is where the story became interesting, revealing two surprises.

The authors solved the crystal structure of the IRE1:ADP:quercetin (Q) complex, which confirmed a new site of small-molecule regulation of IRE1 at the dimer interface of the IRE1 nuclease domain. In the previously solved structures of IRE1, no endogenous ligands bound to this site were captured, highlighting a benefit of screening chemical libraries for new regulators. An unusual feature of quercetin binding was apparent from the crystal structure: two quercetins were bound in one pocket formed at the interface between two IRE1 protomers (Figure 1A).

While it is not unheard of to find two identical drugs bound in the same protein pocket, it is certainly unusual. The first examples of similar binding modes of which I'm aware are the DNA-binding natural products distamycin (Figure 1B) (Pelton and Wemmer, 1989) and chromomycin A (Figure 1C) (Gao and Patel, 1989). The latter is structurally related to quercetin by virtue of a polyhydroxylated chromophore in its central core. Very few examples of the binding of two drugs per binding site are known because such binding is entropically disfavored compared to binding of one drug. Chromomycin solves this problem by using several hydroxyl groups as ligands for Mg^{2+}, which dimerizes the drug in solution even in the absence of DNA. This clever trick of nature for preorganizing a DNA binder is rare in rational ligand design. The oligopeptide antibiotic distamycin similarly binds in a 2:1 complex to the minor groove of DNA, but does not utilize a metal coordination. Instead, drug-drug stacking between pyrroles of one distamycin and amides in its neighbor help pay back the entropic cost of binding two drugs simultaneously. The Q-dimer structure reported by Sicheri and Ron similarly reveals face-to-face stacking of two quercetin molecules. In each of these three examples, drug-drug contacts are likely critical for supporting their ability to bind in a 2:1 fashion.

Many of the most interesting and challenging drug targets today are at protein-protein interfaces. A fundamental challenge in targeting such large surfaces is the need to develop molecules that interact with large surfaces of the target protein. Such molecules are often

between 500 and 800 Da. Although several examples of such large MW compounds are currently in clinical trials, there is a large amount of historical data from drug development to suggest that the smaller the MW of a molecule, the increasing likelihood that it will become approved (Wenlock et al., 2003). Although there are many factors that enter into such a broad characterization of the relationship between MW and likelihood of therapeutic success, the Q-dimer and a reevaluation of the minor groove binders suggest a new approach. What if ligands that assemble on the target by virtue of both drug-protein as well as complementary drug-drug interactions could allow small (<400 Da) molecules to target large protein surfaces? A particularly fruitful class of protein surfaces might be those with two-fold symmetry, like the IRE1 dimer. This direction might also require a reconsideration of the types of functionalities that are normally excluded in medicinal chemistry efforts: in this regard, it is telling that all three examples of two drugs binding simultaneously are natural products, which break most ''rules'' of medicinal chemistry.

Besides the implications for difficult-to-drug protein sites, the new quercetin-binding site reveals new functional inputs into IRE1 regulation. In biochemical assays, quercetin can further enhance IRE1's RNase activity above that elicited by ADP binding to the kinase domain. It is exciting to imagine that an endogenous ligand such as cholesterol, modeled by the authors, could be an in vivo regulator of IRE1 through binding to the new site. Perhaps IRE1 is sensitive to energy status (ADP) as well as to cholesterol or other small molecules that integrate multiple aspects of the cellular protein-folding status.

Finally, different diseases may call for mechanistically distinct classes of IRE1 modulators. In this respect, the new site of IRE1 regulation offers an important new chemical handle for regulation. By exploitation of a mutant IRE1 (D797A) that is resistant to ADP-mediated activation, the authors show that quercetin binding can still activate the RNase activity of the IRE1 mutant. Even more interestingly, when quercetin is bound to the D797A mutant, the enzyme becomes *inhibited* by addition of ADP. This reveals three interesting aspects for future study. First, it suggests that the Q-site and ADP site are not simply independent inputs to RNase activation. Second, it suggests a way to develop a much-needed antagonist of IRE1 RNase activity. This point is critical for therapeutic aspects of IRE1 regulators because in many diseases, such as multiple myeloma, an antagonist of the IRE1-XBP1 pathway is desirable. Lastly, the possibility of regulating the RNase activity independently of the kinase domain of IRE1 through the Q-site could open up more finely tuned ways to modulate the UPR through IRE1.

The work by Sicheri and Ron provides many new avenues to explore in the search for new cellular inputs into IRE1. It also provides a new therapeutic avenue to development of antagonists of IRE1-XBP1 and, potentially, new ways to think about targeting large protein surfaces by taking advantage of intermolecular drug-drug interactions in protein interfaces.

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